PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 9.1.76345/001	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB 03/03864	International filing date *(day/month/year)* 05.09.2003	Priority date *(day/month/year)* 06.09.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant STATOIL ASA, et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06.04.2004	20.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Ulbrecht, M Telephone No. +49 89 2399-7710

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/03864

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-66	as originally filed

Claims, Numbers

9-24	filed with telefax on 16.08.2004
1-8	received on 22.11.2004 with letter of 19.11.2004

Drawings, Sheets

1/6-6/6	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ - This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

   see separate sheet

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 19-24

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 19 are so unclear that no meaningful opinion could be formed *(specify)*:

   see separate sheet

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 20-24

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

☐ complied with.

☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

☐ all parts.

☒ the parts relating to claims Nos. 1-18 .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	7,12-14,16
	No:	Claims	1-6,8-11,15,17,18
Inventive step (IS)	Yes:	Claims	13
	No:	Claims	1-12,14-18
Industrial applicability (IA)	Yes:	Claims	1-18
	No:	Claims	

2. Citations and explanations

see separate sheet

Re item I.

The first line of claim 9 is duplicated.

Re item III.

1. Claim 19 refers to a "battery of probes" suitable for performing the method of any of claims 1-16. The term "battery of probes" is not clear and leaves the reader in doubt as to the technical features to which it refers thereby rendering the definition of said claim unclear (Art. 6 PCT). According to the Merriam-Webster dictionary, "a battery" is "a number of similar articles, items, or devices arranged, connected, or used together". Thus, the most likely interpretation of this term, not commonly used in the field of the invention, would be as referring to "a kit". However, no technical features apart from a result to be achieved definition of the probes is given. No indication of the number of probes whatsoever is contained neither in the claims nor in the passage of the description referring to the said "battery of probes". Moreover, the result to be achieved definition of the probes, namely of being suitable for use in a method according to claims 1-16 does not imply any structural features of these probes. Hence, the claimed "battery of probes" is not defined (Art. 6 PCT).

2. In consequence of the foregoing considerations, no opinion with respect to novelty, inventive step and industrial applicability will be given on claim 19.

Re item IV.

1. The International Preliminary Examination Authority (IPEA) concurs with the objection of the International Search Authority (ISA) as to lack of unity of invention and identifies the following inventions:

1.1 Invention 1: claims 1-19 (all completely)
A method of detecting, characterising or monitoring a hydrocarbon zone comprising the genotypic analysis of a sample for the presence of one or more thermophilic or extremophilic microorganisms.

1.2 Invention 2: claims 20-24 (all partially)

An oligonucleotide as defined in SEQ ID No. 5 or a functional equivalent variant thereof; a solid support having attached thereto said oligonucleotide; a kit comprising said oligonucleotide.

1.3 Inventions 3-16: claims 20-24 (all partially)
Idem as invention 2, but each of the inventions 3-16 restricted to one of the SEQ ID Nos. 6-19.

2. The reasons for the lack of unity being as follows:

2.1 The only identifiable technical feature that all inventions have in common is that they refer to the genotypic analysis of eubacteria and archaea. Inventions 2-16 all relate to oligonucleotide probes for the said bacteria, with inventions 2-8 and 10-16 sharing the technical feature of relating to oligonucleotides specific for 16S rDNA sequences. Inventions 3-5 and 15, finally share the feature of relating to oligonucleotides specific for 16S rDNA sequences of sulphur reducing bacteria.

2.2 However, these features cannot represent special technical features in the sense of R. 13.2 PCT as they are known in the art. Orphan et al. (D1) teaches a method in which samples from different oil fields are genotypically analysed for the presence of thermophilic and extremophilic microorganisms by means of sequence analysis of amplified rDNA sequences using oligonucleotide primers specific for rDNA sequences of eubacteria and archaea. DeLong (D2) teaches oligonucleotide primers and probes specific for the 16S rDNA of inter alia eubacteria and archaea. Van Borm et al. (D3) teaches primers for the amplification of rDNA sequences of various groups of bacteria. Teske et al. (D4) teaches oligonucleotide primers and probes for the analysis of 16S rDNA sequences of sulphate-reducing bacteria including Desulfobacterium, Desulfobacter, Desulfovibrio and Desulfobulbus. EP 0502271 (D5) disclsoes oligonucleotide probes specific for 16S rDNA sequences of sulphate-reducing bacteria, namely Desulfovibrio and Desulfotomaculum as well as methods of detecing said bacteria using said oligonucleotides.

2.3 In view of the prior art represented by D1-D5, the problem of the underlying application can be defined as i) the provision of further uses of a genotypic analysis

of samples for the presence of one or more thermophilic or extremophilic microorganism and ii) the provision of further oligonucelotides in particular for use in the analysis of bacterial 16S rRNA sequences.

2.4 Each of the inventions listed above represents an independent solution concerning one of the foregoing problems of the underlying application. Solution 1 is the use of the genotypic analysis of samples for the presence of one or more thermophilic or extremophilic microorganism in a method of detecing, characterising or monitoring a hydrocarbon zone. Each of the solutions 2-16 provides one of the oligonucleotides according to one of SEQ ID Nos. 5-19.

2.5 In view of the fact that methods involving the genotypic analysis of oil field samples for the presence of one or more thermophilic or extremophilic microorganism using primers specific for rDNA sequences of eubacteria and archaea, as well as oligonucleotides specific for 16S rDNA sequences of archaea and eubacteria, including sulfate-reducing bacteria, namely Desulfobacterium, Desulfobacter, Desulfovibrio, Desulfotomaculum and Desulfobulbus are already known from the prior art; due to the essential differences in primary structure of the oligonucleotides suggested by inventions 2-16; and due to the fact that no other technical features can be distinguished which, in the light of the prior art could be regarded as special technical features common to the above solutions, the IPEA is of the opinion that there is no single inventive concept in the sense of R. 13.1 PCT underlying the 16 solutions contained in the present application. Consequently, there is a lack of unity, and different inventions have been formulate as different subjects as done above.

2.6 As the ISA has searched only the first invention (claims 1-19 (all completely)) the IPEA pursuant R. 66.1(e) is limited to the said searched subject-matter.

Re item V.

1. Reference is made to the following document:

D1: Orphan, V.J. et al.: 'Culture-dependent and culture-independent characterization of microbial assemblages associated with high-temperature petroleum reservoirs", Applied and Environmental Microbiology 66(2):700-711, 2000,

February 2000.

2.1 D1 discloses a method in which samples from different oil fields are genotypically analysed with respect to their microbial content, including the detection of thermophilic and extremophilic microorganisms (abstract; p. 701, c. 1, para. 2 - p. 705, c. 1, para 1; Table 1). The data are represented in relation to the different oil fields (cf. Table 1), thereby characterising each of the said oil fields with respect to its microbial content. As the term characterising a hydrocarbon zone as used in claim 1 is not restricted to the characterisation of the hydrocarbon zone with respect to features other than the microbial content, the subject-matter of claim 1 lacks novelty over D1 (Art. 33(2) PCT). Should the term characterisation be limited to a correlation of one or more target microbes with the properties of the hydrocarbons in question as suggested for one embodiment falling under a method of characterising a hydrocarbon zone according to the invention (cf. p. 7, l. 9-11) this should be reflected in the claim. Moreover, as derivable from the examples of the present application by which oil wells/reservoirs are only characterised with respect to their microbial content, the interpretation of the term "characterisation" used in the examination of claim 1 appears to be justified.

2.2 The method of D1 is considered to generate a microbial profile of the oil filed samples analysed (supra). Furthermore, the said method involves the use of oligonucleotide probes. Hence, the subject-matter of claim 17 also lacks novelty over D1 (Art. 33(2) PCT).

2.3 The additional features suggested by claims 2-4, 6, 9, 11, 15, 17 and 18 are also disclosed in D1 (supra) and thus do not establish novelty over D1. In D1 Thermatogales and Petrotoga as well as Thermotoga, the latter two belonging to the order of Thermotogales are detected. Claim 8 is therefore not novel. The foregoing bacteria are detected by PCR amplification of their rDNA sequences. The primers used for said amplification, thus, must have hybridised to the said sequences of said thermophilic or extremophilic microorganisms. Hence, the additional feature proposed by claim 10 does not establish novelty over D1. In summary, the subject-matter of claims 2-4, 6, 8-11, 15, 17 and 18 lacks novelty over D1 (Art. 33(2) PCT).

2.4 The features suggested by claim 5 do not relate to a method of detecting,

characterising or monitoring a hydrocarbon zone, but to a different method of exploration or production and thus do not establish novelty of the said former method over D1 (Art. 33(3) PCT).

2.5 The subject-matter of claims 7, 12-14 and 16 is novel over the prior art which does not disclose the combination of features suggested by the said claims (Art. 33(2) PCT).

3.1 The oligonucleotide probes suggested by claim 16 include oligonucleotides specific for the 16S rDNA of eubacteria or of archaea. In D1 oligonucleotides with the same specificity, but consisting of different sequences are used. The objective technical problem, thus, consists in providing alternative primers with the same said specificity. In view of this technical problem, the primers proposed by claim 15 are arbitrary selections form equally likely alternatives which do not produce any unforeseeable technical effect beyond that achieved by the oligonucleotides of D1. Hence, the subject-matter of claim 16 does not involve an inventive step (Art. 33(3) PCT).

3.2 The additional feature proposed by claims 7 relates to routine modifications of the method of D1 which do not result in any unforeseeable technical effect and, thus, do not establish an inventive step (Art. 33(3) PCT).

3.3 The method of claim 12 is only supported insofar as it relates to characterising the hydrocarbon zone with respect to the sulphur content and the quality of the oil. No support is present in the description for obtaining information on the type of oil, on the quantity of oil, on the presence of gas or on the gas:oil ratio based on a genotypic analysis of a sample for the presence of thermophilic or extremophilic microorganisms. Hence, inasmuch as claim 12 concerns the said unsupported alternatives claimed, the problem is not considered to be solved and no inventive step can be acknowledged for the claim as a whole (Art. 33(3) PCT).

3.4 As also the method of claim 14 is not supported by the description no inventive step can be acknowledged for its subject-matter (Art. 33(3) PCT).

3.5 Claim 13 provides a method of characterising a hydrocarbon zone in respect of the depth of a hydrocarbon zone. The solution is based on the identification of

microorganisms indicative of a certain depth. No combination of prior art documents neither suggests a method of characterising a hydrocarbon zone with respect its depth nor teaches a correlation of the presence of microorganisms with the depth of the hydrocarbon zone. Hence, claim 13 is considered to involve an inventive step (Art. 33(3) PCT).

4.1 The term "hydrocarbon zone" used in claim 1 is vague and unclear, and leaves the reader in doubt as to the technical feature to which it refers thereby rendering the scope of said claim unclear (Art. 6 PCT). Although, the term "hydrocarbon" is defined at page 6, lines 18-22, the meaning of zone in the context of hydrocarbon is not clear. For the purpose of examination, the term "hydrocarbon zone" was interpreted as referring to Ia. an oil field.

4.2 It was not clear which bacteria fall under the tern "Thermodesulforamonas" as used in claim 8. Said term could not be found in any database (Art. 6 PCT).

Claims

1. A method of detecting, characterising or monitoring a hydrocarbon zone, which method comprises the genotypic analysis of a sample for the presence of one or more thermophilic or extremophilic microorganisms.

2. A method as claimed in claim 1 wherein the sample is from the sub-surface formation.

3. A method as claimed in claim 1 or claim 2 wherein the sample is oil, water or a oil/water mixture from an exploration or production well.

4. A method as claimed in any preceding claim wherein the sample is oil or water that has been exposed to oil.

5. A method as claimed in any preceding claim wherein information regarding the microorganisms present is utilised in an ongoing exploration or production process.

6. A method as claimed in any preceding claim wherein a plurality of different microorganisms are detected thereby generating a microbiological profile for said sample.

7. A method as claimed in claim 6 wherein the generated microbiological profile is compared against one or more reference profiles.

8. A method as claimed in any preceding claim wherein the sample is analysed for the presence of Archaeglobus, Erythrobacter, Arcobacter, Geothermobacter, Thermodesulforamonas and Thermotogales.

9. A method as claimed in any preceding claim which

9. A method as claimed in any preceding claim which does not comprise a culturing step.

10. A method as claimed in any preceding claim wherein the sample is contacted with one or more different oligonucleotides designed to hybridise to regions of nucleic acid from or derived from the thermophilic or extremophilic microorganisms.

11. A method as claimed in any preceding claim wherein at least some of the nucleic acid within the sample is amplified.

12. A method as claimed in any preceding claim wherein the hydrocarbon zone is characterised such that information about the type of oil, the quantity of oil, the quality of the oil, the sulphur content of the oil, the presence of gas or the gas:oil ratio is obtained.

13. A method as claimed in any one of claims 1 to 11 wherein the hydrocarbon zone is characterised such that the depth of a hydrocarbon zone is determined, wherein the particular microorganisms identified are indicative of a certain depth.

14. A method as claimed in any one of claims 1 to 11 wherein the hydrocarbon zone is characterised such that the migration route of said hydrocarbon zone is determined.

15. A method as claimed in claim 13 or claim 14 wherein the hydrocarbon zone is an oil reservoir.

16. A method as claimed in any preceding claim wherein genotypic analysis is performed using one or more probes from the group represented by SEQ ID No. 1 to SEQ ID No. 19.

17. The use of one or more oligonucleotide probes preferably a battery of probes in the generation of a microbiological profile of a sample as defined in any one of claims 2 to 4, wherein said profile is for detcting, characterising or monitoring a hydrocarbon zone.

18. The use of claim 17 wherein said microbiological profile is a pattern which can be compared with a reference sample.

19. A battery of probes for use in the method of any one of claims 1 to 16.

20. An oligonucleotide as defined in any one of SEQ ID No. 5 to SEQ ID No. 19 or a functionally equivalent variant thereof.

21. A solid support having attached thereto one or more oligonucleotides as defined in claim 20.

22. A solid support as claimed in claim 21 which is a microchip.

23. A solid support as claimed in claim 21 or 22 which has at least 4 oligonucleotides as defined in claim 20 attached thereto.

24. A kit for use in a method as claimed in any one of claims 1 to 16 comprising one or more oligonucleotides as defined in claim 20.